

CLAIMS

1. A monoclonal antibody which specifically reacts with a Fas ligand, or an active fragment thereof, wherein
5 the inhibitory activity against apoptosis is higher than that of a Fas-Ig chimera molecule.
2. The monoclonal antibody or the active fragment thereof according to Claim 1, which exhibits higher inhibitory activity against apoptosis at a concentration
10 (effective concentration) of 0.01-8 µg/ml than the Fas-Ig chimera molecule at the same concentration.
3. A monoclonal antibody which specifically reacts with a Fas ligand, or an active fragment thereof, wherein
15 the antibody can inhibit the apoptosis of Fas-expressed cells induced by a soluble Fas ligand at an apoptosis inhibition rate of at least 90%, said apoptosis inhibition rate meaning a survival rate of target cells, to which an antibody has been added, in a cytotoxic reaction test in which a soluble Fas ligand contained in a 12-fold dilution
20 of a culture supernatant of Fas ligand gene-transfected cells is used as an effector molecule, and on the other hand, Fas gene-transfected cells are used as target cells, and both are reacted in a reaction system of 100 µl in a 96-well plate to determine the survival rate of the target
25 cells after 16 hours using a reagent for detecting viable cell numbers.
4. The monoclonal antibody or the active fragment

thereof according to Claim 3, wherein the survival rate (i.e., apoptosis inhibition rate) of the target cells can be enhanced to at least 90% when the soluble Fas ligand contained in the 12-fold dilution of the culture supernatant of the Fas ligand gene-transfected cells is used as the effector molecule in an amount of 25 µl in terms of such a dilution, the Fas gene-transfected cells (Fas/WR19L) are used as the target cells in an amount of 50 µl in terms of its solution at a concentration of 2 × 10⁵ cells/ml, and a culture supernatant of the hybridoma containing said monoclonal antibody is used in an amount of 25 µl to mix all these components with one another, thereby conducting a reaction at 37°C for 16 hours.

5. A monoclonal antibody which specifically reacts with a Fas ligand, or an active fragment thereof, wherein with respect to the inhibition of the physiological reaction between the Fas ligand and Fas, the antibody can inhibit a physiological reaction of a human Fas ligand, but not inhibit a physiological reaction of a mouse Fas ligand.

6. A monoclonal antibody which specifically reacts with a Fas ligand, or an active fragment thereof, wherein the antibody can more strongly react with a Fas ligand than a physiological reaction between the Fas ligand and Fas.

7. A monoclonal antibody which specifically reacts with a Fas ligand, or an active fragment thereof, wherein

the antibody does not react with a mouse-derived Fas ligand classified in the same type as the type of MHC class II of a mouse immunosensitized with a Fas ligand for the purpose of providing said antibody.

5 8. A monoclonal antibody which specifically reacts with a Fas ligand, or an active fragment thereof, wherein the antibody can recognize a Fas ligand present on a human cell surface or a soluble Fas ligand and also a Fas ligand present on a monkey cell surface.

10 9. A monoclonal antibody which specifically reacts with a Fas ligand, or an active fragment thereof, wherein the antibody is produced by a process comprising the steps of (1) immunosensitizing an animal (excluding the human), which does not express a functional Fas molecule, with a 15 Fas ligand molecule or Fas ligand-expressed cells, (2) preparing antibody-producing cells from the immunosensitized animal to form a suspension of the antibody-producing cells, (3) mixing the suspension of the antibody-producing cells with myeloma cells to fuse both 20 cells, (4) diluting the fused cells with a medium which does not favor unfused myeloma cells to culture the fused cells, thereby sorting hybridomas produced by the fusion of the antibody-producing cells with the myeloma cells, (5) determining whether antibodies secreted in a culture 25 supernatant containing the hybridomas are against the desired antigen or not using, as an indicator, the fact that the antibodies inhibit the attack of a Fas ligand

present in a supernatant of Fas ligand-expressed COS cells against Fas-expressed cells, (6) cloning a series of cells in culture wells in which cells secreting the desired antibodies exist, (7) selecting a clone from which the 5 desired antibody is secreted, (8) conducting cloning again to establish a hybridoma clone which secretes a monoclonal antibody against the desired antigen, and (9) preparing the monoclonal antibody from a culture supernatant of the hybridoma or ascites fluid obtained by intraperitoneally 10 administering the hybridoma to a mouse.

10. The monoclonal antibody or the active fragment thereof according to Claim 9, wherein the animal is a rodent belonging to MRL lpr/lpr mice.

11. The monoclonal antibody or the active fragment 15 thereof according to Claim 9, wherein the animal is a rodent belonging to MRL gld mice.

12. A monoclonal antibody which specifically reacts with a Fas ligand, or an active fragment thereof, wherein the antibody reacts with an amino acid sequence region set 20 forth in SEQ ID NO:31 of SEQUENCE LISTING in the Fas ligand.

13. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the 25 inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK1 deposited as Accession No. FERM BP-5044 in National Institute of Bioscience and

Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the H chain consisting of the amino acid sequence set forth in SEQ ID NO:1 of SEQUENCE LISTING.

- 5 14. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK1 deposited as Accession
- 10 No. FERM BP-5044 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the L chain consisting of the amino acid sequence set forth in SEQ ID NO:3 of SEQUENCE LISTING.
- 15 15. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK2 deposited as Accession
- 20 No. FERM BP-5045 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the H chain consisting of the amino acid sequence set forth in SEQ ID NO:5 of SEQUENCE LISTING.
- 25 16. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the

inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK2 deposited as Accession No. FERM BP-5045 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the L chain consisting of the amino acid sequence set forth in SEQ ID NO:7 of SEQUENCE LISTING.

17. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK3 deposited as Accession No. FERM BP-5046 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the H chain consisting of the amino acid sequence set forth in SEQ ID NO:9 of SEQUENCE LISTING.

18. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK4 deposited as Accession No. FERM BP-5047 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the H chain consisting of the amino acid sequence set forth in SEQ ID NO:11 of SEQUENCE LISTING.

19. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an 5 antibody produced by Hybridoma NOK4 deposited as Accession No. FERM BP-5047 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the L chain consisting of the amino acid sequence set forth in SEQ ID 10 NO:13 of SEQUENCE LISTING.

20. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an 15 antibody produced by Hybridoma NOK5 deposited as Accession No. FERM BP-5048 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the H chain consisting of the amino acid sequence set forth in SEQ ID 20 NO:15 of SEQUENCE LISTING.

21. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an 25 antibody produced by Hybridoma NOK5 deposited as Accession No. FERM BP-5048 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and

Technology; and (2) the variable region of the L chain consisting of the amino acid sequence set forth in SEQ ID NO:17 of SEQUENCE LISTING.

22. Mutants of the monoclonal antibody or the active
5 fragment thereof according to any one of Claims 13 to 21.

23. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an
10 antibody produced by Hybridoma NOK1 deposited as Accession No. FERM BP-5044 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the H chain consisting of the amino acid sequence set forth in SEQ ID
15 NO:19 of SEQUENCE LISTING.

24. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an
20 antibody produced by Hybridoma NOK1 deposited as Accession No. FERM BP-5044 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the L chain consisting of the amino acid sequence set forth in SEQ ID
25 NO:21 of SEQUENCE LISTING.

25. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof,

wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK2 deposited as Accession No. FERM BP-5045 in National Institute of Bioscience and
5 Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the H chain consisting of the amino acid sequence set forth in SEQ ID NO:23 of SEQUENCE LISTING.

26. A monoclonal antibody which specifically reacts
10 with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK2 deposited as Accession No. FERM BP-5045 in National Institute of Bioscience and
15 Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the L chain consisting of the amino acid sequence set forth in SEQ ID NO:25 of SEQUENCE LISTING.

27. A monoclonal antibody which specifically reacts
20 with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK3 deposited as Accession No. FERM BP-5046 in National Institute of Bioscience and
25 Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the H chain consisting of the amino acid sequence set forth in SEQ ID

NO:27 of SEQUENCE LISTING.

28. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK3 deposited as Accession No. FERM BP-5046 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the variable region of the L chain consisting of the amino acid sequence set forth in SEQ ID NO:29 of SEQUENCE LISTING.

29. DNAs or RNAs comprising at least a portion encoding the variable region of the H chain or L chain in the monoclonal antibody or the active fragment thereof according to any one of Claims 13 to 28.

30. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK1 deposited as Accession No. FERM BP-5044 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the hypervariable regions of the H chain extending ① from Ser of the 30th to Asn of the 34th, ② from Arg of the 49th to Gly of the 65th and ③ from Tyr of the 93th or Ser of the 98th to Tyr of the 109th of the amino acid sequence set forth in SEQ ID NO:1 of SEQUENCE

LISTING.

31. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK1 deposited as Accession No. FERM BP-5044 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the hypervariable regions of the L chain extending ① from Arg of the 24th to Asn of the 34th, ② from Tyr of the 50th to Ser of the 56th and ③ from Gln of the 89th to Thr of the 97th of the amino acid sequence set forth in SEQ ID NO:3 of SEQUENCE LISTING.
32. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK2 deposited as Accession No. FERM BP-5045 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the hypervariable regions of the H chain extending ① from Asn of the 30th to Gly of the 34th, ② from Tyr of the 49th to Gly of the 65th and ③ from Tyr of the 93th or Tyr of the 98th to Tyr of the 107th of the amino acid sequence set forth in SEQ ID NO:5 of SEQUENCE LISTING.
33. A monoclonal antibody which specifically reacts

with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK2 deposited as Accession

- 5 No. FERM BP-5045 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the hypervariable regions of the L chain extending ① from Lys of the 24th to Gly of the 39th, ② from Leu of the 55th to Ser of the 61th and ③ from Phe
10 of the 94th or Gln of the 95th to Thr of the 102th of the amino acid sequence set forth in SEQ ID NO:7 of SEQUENCE LISTING.

34. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK3 deposited as Accession No. FERM BP-5046 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and
15 Technology; and (2) the hypervariable regions of the H chain extending ① from Ser of the 30th to Asn of the 34th, ② from Arg of the 49th to Gly of the 65th and ③ from Tyr of the 93th or Asp of the 98th to Val of the 105th of the
20 amino acid sequence set forth in SEQ ID NO:9 of SEQUENCE LISTING.

35. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof,

wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK3 deposited as Accession No. FERM BP-5046 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the hypervariable regions of the L chain extending ① from Lys of the 24th to Ser of the 34th, ② from Gly of the 50th to Thr of the 56th and ③ from Val of the 89th or Gln of the 90th to Thr of the 97th of the amino acid sequence set forth in SEQ ID NO:29 of SEQUENCE LISTING.

36. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK4 deposited as Accession No. FERM BP-5047 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the hypervariable regions of the H chain extending ① from Tyr of the 32th to Asn of the 35th, ② from Tyr of the 50th to Asn of the 65th and ③ from Tyr of the 93th to Tyr of the 107th of the amino acid sequence set forth in SEQ ID NO:11 of SEQUENCE LISTING.

37. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an

antibody produced by Hybridoma NOK4 deposited as Accession No. FERM BP-5047 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the hypervariable regions of the L chain extending ① from Arg of the 24th to His of the 38th, ② from Arg of the 54th to Ser of the 60th and ③ from Gln of the 93th to Thr of the 101th of the amino acid sequence set forth in SEQ ID NO:13 of SEQUENCE LISTING.

38. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK5 deposited as Accession No. FERM BP-5048 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology; and (2) the hypervariable regions of the H chain extending ① from Thr of the 30th to His of the 34th, ② from Tyr of the 49th to Asp of the 65th and ③ from Tyr of the 93th to Tyr of the 106th of the amino acid sequence set forth in SEQ ID NO:15 of SEQUENCE LISTING.

39. A monoclonal antibody which specifically reacts with a human Fas ligand, or an active fragment thereof, wherein the antibody has the following features: (1) the inhibitory effect on apoptosis being equal to that of an antibody produced by Hybridoma NOK5 deposited as Accession No. FERM BP-5048 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and

Technology; and (2) the hypervariable regions of the L chain extending ① from Lys of the 24th to Ala of the 34th, ② from Tyr of the 50th to Thr of the 56th and ③ from Gln of the 89th to Thr of the 97th of the amino acid sequence 5 set forth in SEQ ID NO:17 of SEQUENCE LISTING.

40. Mutants of the monoclonal antibody or the active fragment thereof according to any one of Claims 30 to 39.

41. DNAs or RNAs comprising at least a portion encoding the hypervariable region of the H chain or L 10 chain in the monoclonal antibody or the active fragment thereof according to any one of Claims 30 to 39.

42. A monoclonal antibody which specifically reacts with a Fas ligand, or an active fragment thereof, wherein the antibody is produced by any one of hybridoma cell 15 lines deposited as Accession Nos. FERM BP-5044 (Hybridoma NOK1), FERM BP-5045 (Hybridoma NOK2), FERM BP-5046 (Hybridoma NOK3), FERM BP-5047 (Hybridoma NOK4), FERM BP-5048 (Hybridoma NOK5) and FERM BP-5334 (Hybridoma KAY-10) 20 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology.

43. The monoclonal antibody or the active fragment thereof according to any one of Claims 1, 3, 5, 6, 7, 8, 9 and 12, which can affinity-purify a soluble Fas ligand present in a culture supernatant of Fas ligand-expressed 25 cells.

44. The monoclonal antibody or the active fragment thereof according to any one of Claims 1, 3, 5, 6, 7, 8, 9

and 12, which can immunoprecipitate Fas ligand molecules on Fas ligand-expressed cell surfaces or soluble Fas ligand molecules secreted in a culture solution.

45. A process for producing monoclonal antibodies
- 5 specifically reacting with a Fas ligand, which comprises the steps of (1) immunosensitizing an animal (excluding the human), which does not express a functional Fas molecule, with a Fas ligand molecule or Fas ligand-expressed cells, (2) preparing antibody-producing cells
- 10 from the immunosensitized animal to form a suspension of the antibody-producing cells, (3) mixing the suspension of the antibody-producing cells with myeloma cells to fuse both cells, (4) diluting the fused cells with a medium which does not favor unfused myeloma cells to culture the
- 15 fused cells, thereby sorting hybridomas produced by the fusion of the antibody-producing cells with the myeloma cells, (5) determining whether antibodies secreted in a culture supernatant containing the hybridomas are against the desired antigen or not using, as an indicator, the
- 20 fact that the antibodies inhibit the attack of a Fas ligand present in a supernatant of Fas ligand-expressed COS cells against Fas-expressed cells, (6) cloning a series of cells in culture wells in which cells secreting the desired antibodies exist, (7) selecting a clone from
- 25 which the desired antibody is secreted, (8) conducting cloning again to establish a hybridoma clone which secretes a monoclonal antibody against the desired antigen,

and (9) preparing the monoclonal antibody from a culture supernatant of the hybridoma or ascites fluid obtained by intraperitoneally administering the hybridoma to a mouse.

46. A method of detecting a Fas ligand in a solution,
5 which comprises combining a plurality of monoclonal antibodies against Fas ligand according to any one of Claims 1 to 28, 30 to 40, and 42 to 44 with each other.

47. The detection method according to Claim 46,
wherein one of the plural monoclonal antibodies is
10 immobilized on a carrier, the other monoclonal antibody is labeled with a labeled compound, the carrier on which the monoclonal antibody has been immobilized is brought into contact with a solution of a specimen which is considered to contain a Fas ligand, thereby adsorbing the specimen,
15 and the adsorbed specimen is detected by the monoclonal antibody labeled with the labeled compound.

48. The detection method according to Claim 47,
wherein a purified monoclonal antibody of IgM type is
immobilized on a carrier, and a Fas ligand in a solution
20 is detected by a biotin-labeled monoclonal antibody of IgG type.

49. A kit for use in detecting a Fas ligand,
comprising in combination a plurality of monoclonal antibodies against Fas ligand according to any one of
25 Claims 1 to 28, 30 to 40, and 42 to 44.

50. The kit according to Claim 49, which detects a concentration of a Fas ligand in the blood of a person

attacked by infectious mononucleosis (IM), systemic lupus erythematoses (SLE) or hepatitis.

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